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(54) Title: CHIMERIC INFECTIOUS BURSAL DISEASE VIRUS cDNA CLONES, EXPRESSION PRODUCTS AND VACCINES BASED THEREON					
(57) Abstract					
Chimeric cDNA for the expression of immunogenic polypeptides include the genetic epitopic determinants for a base infectious bursal disease virus strain and at least one other infectious bursal disease virus strain. The genetic epitopic determinants encode amino acids or amino acid sequences which define epitopes bound to by previously established monoclonal antibodies. The immunogens expressed by the cDNA may be employed to provide a vaccine against a plurality of IBDV strains. The epitopic determinant of IBDV lethal strains has been detected, and an immunogen for conferring immunity with respect thereto is disclosed. Similarly, a monoclonal antibody specific for IBDV lethal strains is identified, and a vaccine for passive immunization therewith is also disclosed. Immunogens exhibiting conformational epitopes, in the form of virus-like particles, are effective in the preparation of vaccines.					

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Description

Chimeric Infectious Bursal Disease Virus cDNA Clones,  
Expression Products and Vaccines Based Thereon

Technical Field:

The present invention provides chimeric IBDV immunogens which actively protect against virulent and lethal challenge by Classic and variant IBDV strains, and methods for obtaining vaccines containing these chimeric immunogens and vaccines.

Background Art

Infectious bursal disease virus (IBDV) is responsible for a highly contagious immunosuppressive disease in young chickens which causes significant losses to the poultry industry worldwide (reviewed in Kibenge (1988) "J. Gen. Virol.", 69:1757-1775). Infection of susceptible chickens with virulent IBDV strains can lead to a highly contagious immunosuppressive condition known as infectious bursal disease (IBD). Damage caused to the lymphoid follicles of the bursa of Fabricius and spleen can exacerbate infections caused by other agents and reduce a chicken's ability to respond to vaccination as well (Cosgrove (1962) "Avian Dis.", 6:385-3894).

There are two serotypes of IBDV (McFerran et al (1980) "Avian Path." 9:395-404). Serotype 1 viruses are pathogenic to chickens and differ markedly in their virulence (Winterfield et al (1978) "Avian Dis." 5:253-260), whereas serotype 2 viruses, isolated from turkeys, are avirulent for chickens (Ismail et al (1988) "Avian Dis.", 32:757-759; Kibenge (1991) "Virology" 184:437-440).

IBDV is a member of the Birnaviridae family and its genome consists of two segments of double-stranded RNA (Dobos et al (1979) "J. Virol.", 32:593-605). The smaller segment B (~2800bp) encodes VP1, the dsRNA polymerase. The larger genomic segment A (~3000bp) encodes a 110 kDa precursor polyprotein in a single open reading frame (ORF) that is processed into mature VP2, VP3 and VP4 (Azad et al (1985)

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"Virology" 143:35-44). From a small ORF partly overlapping with the polyprotein ORF, segment A can also encode VP5, a 17 Kda protein of unknown function (Kibenge et al (1991) "J. Gen. Virol.", 71:569-577).

While VP2 and VP3 are the major structural proteins of the virion, VP2 is the major host-protective immunogen and causes induction of neutralizing antibodies (Becht et al (1988) "J. Gen. Virol." 69:631-640; Fahey et al (1989) "J. Gen. Virol.", 70:1473-1481). VP3 is considered to be a group-specific antigen because it is recognized by monoclonal antibodies (Mabs) directed against VP3 from strains of both serotype 1 and 2 (Becht et al (1988) "J. Gen. Virol.", 69:631-640). VP4 is a virus-coded protease and is involved in the processing of the precursor protein (Jagadish et al (1988) "J. Virol.", 62: 1084-1087).

In the past, control of IBDV infection in young chickens has been achieved by live vaccination with avirulent strains, or principally by the transfer of maternal antibody induced by the administration of live and killed IBDV vaccines to breeder hens. Unfortunately, in recent years, virulent variant strains of IBDV have been isolated from vaccinated flocks in the United States (Snyder et al (1988b) "Avian Dis.", 32:535-539; Van der Marel et al (1990) "Dtsch. Tierarztl. Wschr.", 97:81-83). The use of a select panel of Mabs, raised against various strains of IBDV, has led to the identification of naturally occurring GLS, DS326, RS593 and Delaware variant viruses in the United States. Substantial economic losses have been sustained due to the emergence of these antigenic variants (Delaware and GLS) in the field (Snyder et al (1992) "Arch. Virol.", 127:89-101), copending U.S. Application Serial No. 08/216,841, filed March 24, 1994, Attorney Docket No. 2747-053-27, Snyder, copending herewith). These variant strains are antigenically different from the Classic strains of IBDV most typically isolated before 1985, and lack epitope(s) defined by neutralizing monoclonal antibodies

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(Mabs) B69 and R63 (Snyder et al (1988a) "Avian Dis.", 32:527-534; Snyder et al (1998b) "Avian Dis.", 32:535-539; Snyder et al (1992) "Arch. Virol.", 127:89-101). Since the appearance of these variant strains in the field, many commercially available live and killed vaccines for IBDV have been reformulated in an attempt to better match the greater antigenic spectrum of viruses recognized to be circulating in the field.

Efforts to develop a recombinant vaccine for IBDV have been made, and the genome of IBDV has been cloned (Azad et al (1985) "Virology", 143:35-44). The VP2 gene of IBDV has been cloned and expressed in yeast (Macreadie et al (1990) "Vaccine", 8:549-552), as well as in a recombinant fowlpox virus (Bayliss et al (1991) "Arch. Virol.", 120:193-205). When chickens were immunized with the VP2 antigen expressed from yeast, antisera afforded passive protection in chickens against IBDV infection. When used in active immunization studies, the fowlpox virus-vectored VP2 antigen afforded protection against mortality, but not against damage to the bursa of Fabricius.

Recently, the synthesis of VP2, VP3 and VP4 structural proteins of the variant GLS IBDV strain in a *baculovirus* expression system has been described (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In an initial two dose active immunity study in SPF chickens, *baculovirus* expressed GLS proteins were able to confer 79% protection against virulent GLS challenge (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In a subsequent extended study of active cross-immunity, by increasing the antigenic mass of the *baculovirus* expressed GLS protein, complete protection against the variant GLS and E/Del strains was obtained with a single dose, but only partial protection was afforded against the Classic STC strain unless two doses were administered.

In recent years, the complete, nucleotide sequences of the large segment A of five serotype 1 IBDV strains; 002-73

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(Hudson et al (1986) "Nucleic Acids Res." 14:001-5012), Cu-1, PBG98, 52/70 (Bayliss et al (1990) "J. Gen. Virol.", 71:1303-1312), STC (Kibenge (1990) "J. Gen. Virol.", 71:569-577), and serotype 2 OH strain (Kibenge (1991) "Virology", 184:437-440) have been determined. In addition, the VP2 gene of virulent Japanese IBDV strains (Lin et al (1993) "Avian Dis.", 37:315-323) and Delaware variants A and E (Lana et al (1992) "Virus Genes" 6:247-259; Heine et al (1991) "J. Gen. Virol.", 22:1835-1843) has been sequenced. However, noone has completely cloned and characterized the entire long segment of any United States IBDV variant.

#### Disclosure of the Invention

Inventors have now identified the region of the IBDV genome which is responsible for antigenic variation. A DNA sequence containing the central variable region of VP2 protein, as well as a plasmid incorporating the same, have been constructed. This DNA sequence can be manipulated to generate desired virus neutralizing epitopes or immunogenic polypeptides of any IBDV strain. In turn, these immunogenic segments can be incorporated into new recombinant IPDV vaccines.

#### Brief Description of the Drawings

Figure 1 illustrates the construction of various chimeric plasmids encoding IBDV-specific polyproteins. A map of the IBDV genome with its coding regions is shown at the top of the Figure. Selected restriction sites are incorporated in the Figure: B, *Bam*HI; E, *Bst*EIII; N, *Nde*I; R, *Nar*I; S, *Spe*I. Dashes indicated the substitution of the D78 sequence (*Nde*I-*Nar*I fragment) into the GLS sequence to restore the B69 epitope region. Solid line and dotted line indicate the substitution of the E/De1-22 and DS326 sequences, respectively, into the GLS sequence to restore the B63 epitope region or to delete the 179 epitope region, respectively.

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Figure 2 is electron micrographs of IBDV virus-like particles (—) = 100nm). A. Actual empty particles (without RNA) from purified virus. B. Virus-like particles (empty capsids) derived from a recombinant baculovirus expressing the large genome segment of IBDV in insect cells

Figure 3 is a comparison of the deduced amino acid sequences of the structural proteins (VP2, VP3 and VP4) of ten IBDV strains. Dashes (-) indicate amino acid identity and crosses (x) denote a region where the sequence was not determined. Filled bar (█) indicates a gap in the sequence and vertical arrowheads (↑) mark the possible cleavage sites of VP2/VP4 and VP4/VP3. The two hydrophilic peaks in the variable region are overlined.

Figure 4 is a phylogenetic tree for the IBDV structural proteins using the PAUP (phylogenetic analysis using parsimony) version 3.0 program (Illinois Natural History Survey, Champaign, Illinois).

Figure 5 reflects the DNA and amino acid sequence for the GLS virus structural protein fragment VP2/VP4/VP3. A vertical line indicates the start/stop points for the VP2, VP4 and VP3 regions.

Figure 6 reflects the DNA and amino acid sequence for the E/DeL 22 virus structural protein fragment VP2/VP4/VP3.

Figure 7 is a table of the amino acid identities for key locations (epitopic determinants) of eight different IBDV.

Definitions:

IBD - infectious bursal disease as described above.

IBDV - infectious bursal disease virus, a virus capable of, at a minimum, inducing lesions in the bursa of Fabricius in infected poultry.

Epitopic Determinants - amino acids or amino acid sequences which correspond to epitopes recognized by one or more monoclonal antibodies. Presence of the amino acid or amino acid sequence at the proper ORF location causes the

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polypeptide to exhibit the corresponding epitope. An epitopic determinant is identified by amino acid(s) identity and sequence location.

Genetic Epitopic Determinants - nucleotide sequences of the ORF which encode epitopic determinants.

Conformational Epitopes - epitopes induced, in part or in whole, by the quaternary (three-dimensional) structure of an IBDV polypeptide. Conformational epitopes may strengthen binding between an IBDV and a monoclonal antibody, or induce binding whereas the same sequence, lacking the conformational epitope, would not induce binding between the antibody and the IBDV polypeptide at all.

Virus-Like Particles - three-dimensional particles of natural or recombinant amino acid sequences mimicking the three-dimensional structure of IBDV (encoded by the large genome segment A) but lacking viral RNA. Virus-like particles exhibit conformational epitopes exhibited by native viruses of similar sequence. Virus-like particles are created by the proper expression of DNA encoding VP2, VP4, VP3 structural proteins in a proper ORF.

Epitopic Determinant Region - Limited region of amino acid sequence of VP2 of IBDV that is replete with epitopic determinants, variation among amino acids of this limited region giving rise to a high number of epitopes recognized by different monoclonal antibodies.

#### Best Mode for Carrying Out the Invention

Recombinant, immunogenic polypeptides exhibiting the epitopes of two or more native IBDV, as well as recombinant virus-like particles exhibiting the epitopes of two or more native IBDV and conformational epitopes are effective immunogens for vaccines which can be used to confer protection against a wide variety of IBDV challenge in inoculated poultry. The recombinant polypeptides and virus-like particles are obtained by the expression of chimeric DNA

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prepared by the insertion, in the VP2 region of a base IBDV, of epitopic determinants for at least a second IBDV. This is most easily done by substitution of the genetic epitopic determinants for the amino acids identities and locations reflected in Figure 7. Thus, where the epitopic determinant of the second IBDV differs from that of the base IBDV, the genetic epitopic determinant for the differing second IBDV is inserted in place of the genetic epitopic determinant at that location of the base IBDV. An example, combining epitopic determinants from the D78, E/Del 22 and DS326 IBDV into the base GLS IBDV is set forth in Figure 1. Thus, one DNA sequence can be prepared with genetic epitopic determinants for a plurality of native IBDV. These recombinant plasmids can be inserted into a variety of packaging/expression vector, including *baculovirus*, *fowlpox virus*, *Herpes virus of turkeys*, *adenovirus* and similar transfection vectors. The vectors can be used to infect conventional expression cells, such as SF9 cells, chicken embryo fibroblast cell lines, chicken embryo kidney cells, vero cells and similar expression vehicles. Methods of transfection, and methods of expression, as well as plasmid insertion into transfection vehicles, are well known and do not constitute an aspect of the invention, *per se*.

The expression of the chimeric cDNA of the invention generate immunogenic polypeptides which reflect epitopes of a plurality of native IBDV, and the expression of a recombinant VP2, VP4, VP3 cDNA segment, with the VP2 region again comprising genetic epitopic determinants for at least two native IBDV give rise to immunogenic virus-like particles.

The immunogenic polypeptides and virus-like particles can be harvested using conventional techniques (Dobos et al, "J. Virol.", 32:593-605 (1979)). The polypeptides and virus-like particles can be used to prepare vaccines which will confer protection on inoculated poultry, in particular, chickens, and in a preferred embodiment, broiler chickens, protection against challenge from each IBDV bearing an epitope reflected

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in the plurality of epitopic determinants present in the inoculum. Thus, a single immunogen gives rise to immunity against a variety of IBDV, each IBDV whose genetic epitopic determinant has been incorporated in the chimeric cDNA.

The administration of the vaccines can be effectively done according to well-established procedures. In U.S. Patent 5,064,646, which is incorporated herein by reference, methods are described for the effective inoculation of chicks based on the then novel isolation of GLS IBDV. Similar administration and dosage regimens can be employed herein. Since the polypeptides and virus-like particles lack viral RNA, they are avirulent. The vaccines may therefore be prepared by simple incorporation of the immunogenic polypeptides and virus-like particles in a pharmaceutical carrier, typically a suspension or mixture. Appropriate dosage values are best determined through routine trial and error techniques, given the different antibody titers induced and/or the quantity of different epitopes present which will induce complete cross-immunity to virulent challenge. In general, pharmacologically acceptable carriers such as a phosphate buffered saline, cell culture medium, Marek's virus vaccine diluent oil adjuvants and other adjuvants, etc., can be used. Administration is preferably done to hens entering egg laying periods which provides induction of antibody which is passively transferred through the egg to the chick to prevent early invention by virulent field strength IBDV. Conversely, the recombinant vaccine may be delivered in a replicating vector at any time in a chicken's life span, preferably at one day of age. Experience has demonstrated that, generally, that the level of protection may be improved by a second inoculation.

This invention may be further understood by reference to the specific examples set forth below.

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Examples:

**Background Methodology**

To determine the molecular basis of antigenic variation in IBDV, the genomic segment A of four IBDV strains: GLS, DS326, Delaware variant E (E/Del) and D78 was cloned and characterized by sequencing. By comparing the deduced amino acid sequences of these strains with other serotype 1 and 2 sequences published previously, the putative amino acid residues involved in the binding with various neutralizing Mabs were identified, and the phylogenetic relationship of IBDV structural proteins was examined.

GLS, DS326 and STC strains of IBDV were propagated in the bursa of specific-pathogen-free chickens (SPAFAS, Inc., Norwich, CT, USA). Tissue culture adapted E/Del-22, D78 and OH (serotype 2) strains of IBDV were propagated in primary chicken embryo fibroblast cells derived from 10-day-old embryonated eggs (SPAFAS, Inc.) and purified as described (Snyder et al (1988a) "Avian Dis.", 32:527-534). The Mabs against various strains of IBDV were produced and characterized using protocols previously outlined (Snyder et al (1988a) "Avian Dis." 32:527-534; Snyder et al (1988b) "Avian Dis.", 32:535-539). Mabs B69 and R63 were prepared against D78 strain, whereas Mabs 8, 10, 57 and 179 were prepared against GLS strain. In addition, a new Mab 67 was prepared which was neutralizing and specific for the E/Del strain. Identification of IBDV antigens by modified antigen capture ELISA (AC-ELISA) was carried out as described (Snyder et al (1992) "Arch. Virol.", 127:89-101).

Various strains of IBDV were characterized by their reactivities with a panel of neutralizing Nabs, as shown in Table 1.

TABLE 1  
Antigenic characterization of various IBDV strains by their reactivities with a panel of neutralizing MAbs

Virus Strains	Classification	Reactivities with MAbs					
		B69	R63	179	8	10	57
D78	Classic	+	+	+	+	-	-
PBG98	Classic	-	+	+	+	-	-
STC	Classic	+	+	+	+	-	-
52/70	Classic	+	+	+	-	-	-
OH (serotype 2)	Classic	+	+	+	-	-	-
E/De1	Variant	-	+	+	-	-	+
GLS	Variant	-	-	+	+	+	-
DS326	Variant	-	-	-	+	+	-

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All standard serotype 1 viruses reacted with Mabs B69, R63, 179 and 8, except PBG98 (a British vaccine strain, Intervet, U. K.) which did not react with Mab B69. In contrast, all the U.S. variant viruses lack the virus-neutralizing B69 epitope. In addition, GLS and DS326 variants lack an R63 epitope but share a common epitope defined by the Mab 57. Thus, on the basis of the reactivities with various Mabs, these viruses were antigenically grouped as classic, GLS, DS326 and E/Del variants.

Complementary DNA clones, containing the entire coding region of the large RNA segment of various IBDV strains, were prepared using standard cloning procedures and methods previously described (Vakharia et al (1992) "Avian Dis.", 36:736-742; Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). The complete nucleotide sequence of these cDNA clones was determined by the dideoxy method using a Sequenase DNA sequencing kit (U.S. Biochem. Corp., Columbus, OH). DNA sequences and deduced amino acid sequences were analyzed by a PC/GENE software package (Intelligenetics, Inc.). These are reflected in Figures 5 and 6. The nucleotide sequence data of the GLS strain has been deposited with GenBank Data Libraries and has been assigned an accession number M97346.

Comparisons of the nucleotide sequence of GLS strain (3230 bp long) with eight serotype 1 and one serotype 2 IBDV strains exhibit  $\geq 92\%$  and  $\geq 82\%$  sequence homology, respectively; indicating that these viruses are closely related. It is interesting to find that there are only six to nine base substitutions between D78, PBG98, and Cu1 strains which corresponds to a difference of about 0.2% to 0.3% (results not shown). Figure 3 and Table 2 show a comparison of the deduced amino acid sequences and percent homology of the large ORF of segment A of the ten IBDV strains, including four IBDV strains used in this study.

TABLE 2  
Percent amino acid sequence homology of large ORF of segment A of ten IBDV strains

Strain	GLS	DS326	E/De1	D78	Cu-1	PBG98	52/70	STC	002-73	OH
GLS	98.7									
DS326	98.4	98.3								
E/De1	98.4	98.3								
D78	98.5	98.1	97.9							
Cu-1	98.6	98.2	98.0	99.6						
PBG98	98.5	98.1	97.9	99.5	99.5					
52/70	98.1	98.1	97.9	98.4	98.5	98.5	98.3			
STC	97.7	98.0	97.5	98.4	98.5	98.5	98.3	98.3		
002-73	97.0	97.1	96.7	97.6	97.7	97.7	97.6	97.3	97.4	
OH	90.0	90.0	89.7	90.2	90.3	90.2	89.8	90.3	90.1	

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These comparisons show that the proteins are highly conserved. The degree of difference in the amino acid sequence ranges from 0.4% for the D78 versus Cu-1 comparison and 10.3% for the serotype 1 (E/Del) versus serotype 2 (OH) comparison (Table 2).

In Figure 3, alignments of the deduced amino acid sequences of the large ORF (1012 residues) of ten IBDV strains (including four used in this study) show that most of the amino acid changes occur in the central variable region between residues 213 and 332 of VP2 protein, as shown earlier by Bayliss et al (1990) "J. Gen. Virol. M, 71:1303-1312. It is interesting to note that all the U.S. variants (GLS, DS326 and E/Del) differ from the other strains in the two hydrophilic regions which are overlined in Figure 3 (residues 212 to 223 and residues 314 to 324). These two hydrophilic regions have been shown to be important in the binding of neutralizing Mabs and hence may be involved in the formation of a virus-neutralizing epitope (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843). Recently, we demonstrated that the conformation dependent Mabs B69, R63, 8, 179, 10, and 57 (see Table 2) immunoprecipitate VP2 protein (Snyder et al (1992) "Arch. Virol.", 127:89-101). In addition, E/Del specific Mab 67 also binds to VP2 protein. Therefore, to identify the amino acids involved in the formation of virus-neutralizing epitopes, and hence the antigenic variation, we compared the amino acid sequences of VP2 protein of classic and variant viruses.

Comparison of the D78 sequence with the PBG98 sequence shows only four amino acid substitutions at positions 76, 249, 280 and 326. However, STC and 52/70 strains also differ from the D78 sequence at positions 76, 280 and 326 but these viruses do bind to Mab B69. This implies that Gln at position 249 (Gln249) may be involved in the binding with Mab B69. It should be noted that all U.S. variant viruses have a Gln→Lys substitution at this position and hence escape the binding

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with neutralizing Mab B69. Similarly, comparison of the GLS sequence with the DS326 sequence in the variable region shows six amino acid substitutions at positions 222, 253, 269, 274, 311 and 320. However, other strains of IBDV that do bind to Mab 179 have amino acid substitutions at positions 222, 253, 269 and 274 that are conservative in nature. Therefore, this suggests that Glu311 and Gln320 may be involved in the binding with Mab 179. Again, comparison of GLS and DS326 sequences with all other IBDV sequences shows a unique Ala→Glu substitution at position 321, suggesting the contribution of this residue in the binding with Mab 57. Since Mab 57 does not compete with Mab R63, it is conceivable that Ala321 may contribute to the binding with Mab R63. Similarly, comparison of E/Del sequence with other sequences shows five unique substitutions at positions 213, 286, 309, 318 and 323. However, comparison of this E/Del sequence (from tissue culture derived virus) with previously published VP2 A/Del and E/Del sequences (bursa derived virus) suggests the involvement of Ile286, Asp318 and Glu323 in the binding with Mab 67 since residues at positions 213 and 309 are not substituted in A/Del and E/Del sequences, respectively (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843; Lana et al (1992) "Virus Genes", 6:247-259; Vakharia et al (1992) "Avian Dis.", 36:736-742).

Comparisons of the amino acid sequence also show a striking difference between serotype 1 and serotype 2 sequences. In serotype 2 OH strain, there is an insertion of an amino acid residue at position 249 (serine) and a deletion of a residue at position 680. Previously, it has been shown that serotype 2 viruses are naturally avirulent and do not cause any pathological lesions in chickens (Ismail et al (1988) "Avian Dis.", 32:757-759). Thus, these subtle changes in the structural proteins of serotype 2 OH strain may play an important role in the pathogenicity of the virus. Moreover, it has been hypothesized that an amino acid sequence motif, S-W-S-A-S-G-S, (residues 326 to 332) is conserved only in

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virulent strains and could be involved in virulence (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843). This sequence motif was also conserved in various pathogenic strains of IBDV isolated in Japan (Lin et al (1993) "Avian Dis.", 37:315-323). Comparison of the amino acid sequences in this heptapeptide region reveals that nonpathogenic serotype 2 OH strain has three substitutions, whereas mildly pathogenic strains of serotype 1 (D78, Cu-1, PBG98 and 002-73) have one or two substitutions in this region. Moreover, comparison of the hydrophilicity plots of the variable region (amino acids 213 to 332) of variant serotype 1 strains and serotype 2 OH strain indicates a drastic reduction in the second hydrophilic peak region (amino acid residues 314 to 324) for serotype 2 (results not shown). Since most of the amino acid residues causing antigenic variation reside in this region, these residues may play an important role in the formation of virus-neutralizing epitopes, as well as serotype specificity.

To evaluate the antigenic relatedness of structural proteins of various IBDV strains, a phylogenetic tree was constructed, based on the large ORF sequences of ten IBDV strains, including the U.S. variant strains examined in this study (Figure 4). Three distinguishable lineages were formed. The first one, which is most distant from the others, is serotype 2 OH strain, and the second one is the geographically distant Australian serotype 1 strain (002-73). The third lineage consists of four distinct groups. The first and second group include highly pathogenic strains, namely, standard challenge (STC) strain from U. S. and the British field strain (52/70). The third group comprises all the European strains: the vaccine strains D78 (Holland), PBG98 (U.K.), and mildly pathogenic strain Cu-1 (Germany). The fourth group consists of the U.S. variant strains in which E/Del forms a different subgroup. The groups formed by the phylogenetic analysis correlate very well with the Mabs reactivity patterns (see Table 1). As shown in Figure 4, all

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the U.S. variant viruses which lack the B69 epitope form a distinct group, whereas all the classic viruses containing a B69 epitope form another group (except PBG98). In addition, closely related GLS and DS326 strains containing a common Mab 57 epitope and lacking an R63 epitope could be separated from the other variant E/DeL strain.

Based on this information, a recombinant vaccine was constructed as follows:

**Construction of recombinant baculovirus clones containing chimeric IBDV genes**

A recombinant baculovirus which expresses a chimeric VP2, VP3 and VP4 structural proteins of the GLS strain was constructed and assessed. The recombinant baculovirus expressed a chimeric VP2 protein incorporating all Mab defined GLS neutralization sites, as well as one neutralization site (B69) which is specific for Classic strains of IBDV in the form of a VP2-VP4-VP3 segment.

Complementary DNA clones, containing the entire coding region of the large RNA segment of the GLS and D78 IBDV strains, were prepared using standard cloning procedures and methods previously described (Vakharia et al (1992) "Avian Dis.", 36:736-742; Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). To insert the gene sequence encoding the B69 epitope of the D78 IBDV strain, plasmid pB69GLS was constructed as follows (see Figure 1). Full-length cDNA clones of D78 and GLS (plasmids pD78 and pGLS-5) were digested with *Nde*I-*Nar*I and *Nar*I-*Spe*I enzymes to release a *Nde*I-*Nar*I (0.26 kb) and a *Nar*I-*Spe*I (0.28 kb) fragments, respectively. These two fragments were then ligated into the *Nde*I-*Spe*I cut plasmid pGLS-5 to obtain a chimeric plasmid pB69GLS. As a result of this insertion, three amino acids were substituted in the GLS VP2 protein. These substitutions were at positions 222 (Thr-Pro), 249 (Lys-Gln) and 254 (Ser-Gly) in the variable region of the VP2 protein (Vakharia et al (1992) "Avian Dis.",

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36:736-742). To insert the chimeric IBDV structural genes in the *Baculovirus* genome, plasmid pB69GLS was completely digested with *Bst*ECII enzyme and partially with the *Bam*HI enzyme, combined with the *Nhe*I-*Bst*ECII fragment (derived from plasmid pGLSBacI, see Vakharria et al (1993) "J. Gen. Virol.", 74:1201-1206) and then ligated to the *Nhe*I-*Bam*HI cut transfer vector pBlueBacII (Invitrogen Corp., San Diego, CA). Finally, recombinant *baculovirus* I-7 was obtained using previously described procedures (Vakharria et al (1993) "J. Gen. Virol.", 74:1201-1206). See Table 3.

#### **Preparation of an inoculum for immunization**

*Spodoptera frugiperda* SF9 cells, infected at a multiplicity of 5 PFU per cell with the I-7 recombinant *baculovirus*, were propagated as suspension cultures in one liter flasks containing Hink's TNM-FH medium (JHR Biosciences, Lenxa, KS) supplemented with 10% fetal calf serum at 28°C for 3 to 4 days. The infected cells were recovered by low speed centrifugation, washed two times with PBS, and resuspended in a minimum volume of PBS. The cell slurry was sonicated on an ice bath three times for 1 min, with 2 min intervals and clarified by low speed centrifugation. An aliquot of each cell lysate was tested with anti-IBDV Mabs by AC-ELISA to estimate the antigenic mass present (Snyder et al (1998b) "Avian Dis.", 32:535-539). Preparations having the highest antigenic mass were pooled and comparatively titrated in AC-ELISA against the V-IBDV-7-1 recombinant *baculovirus* IBDV vaccine used in a previous study (Vakharria et al (1993) "J. Gen. Virol.", 74:1201-1206). The antigenic mass of the I-7 recombinant preparation, as determined by AC-ELISA with group specific neutralizing Mab 8, was adjusted by dilution to be the same as the V-IBDV-7-1 vaccine, and then it was emulsified with an equal volume of Freund's incomplete adjuvant and used for inoculation.

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### Viruses

The challenge viruses: Classic strains IM and STC, and variant strains E/Del and GLS-5 were obtained from previously acknowledged sources (Snyder et al (1988a) "Avian Dis.", 32:527-534; Snyder et al (1992) "Arch. Virol.", 127:89-101). After intraocular instillation, challenge viruses were titrated in the bursae of specific-pathogen-free (SPF) chickens (SPAFAS, Inc., Storrs, Conn.). For strains STC, E/Del and GLS-5, a 100 chick infective fifty percent dose (100 CID<sub>50</sub>) was determined based on bursa to body weight measurements. One hundred lethal doses (100 LD) of the IM strain were calculated based on mortality at 8 days post-inoculation (PI).

### Chicken inoculations and IBDV challenge

White leghorn SPF chickens were hatched and reared in HEPA filtered isolation units (Monair Anderson, Peachtree City, GA). Eight-week old chickens were prebled, individually wing banded, divided among 10 groups of 15 chicks each and treated as follows. Chickens of groups I-V received no inoculations and served as either negative or positive challenge controls. Chickens of group V-X were inoculated intramuscularly with 0.5 ml of the 1-7 inoculum prepared above from recombinant *Baculovirus* infected cell lysates. At 3 weeks PI, all chickens were bled and chickens of groups II-IX were challenged with the appropriate IBDV challenge strain by ocular instillation. Four days post-challenge, 5 chickens from each group were humanely sacrificed and their cloacal bursa were removed. Each bursa was processed and subsequently evaluated for the presence of IBDV antigen by AC-ELISA as described (Snyder et al (1998b) "Avian Dis.", 32:535-539). In addition, chickens in the IM challenged groups were scored as dead, and humanely sacrificed when they became obviously moribund due to IM challenge. Eight days post-infection, the remaining chickens in all groups were sacrificed and weighed.

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The bursa of Fabricius from each chicken was carefully excised and also weighed. Bursa weight to body weight ratio was calculated for each chicken as described by Lucio and Hitchner (Lucio et al (1979) "Avian Dis.", 23:466-478). Any value for individually challenged chickens falling plus or minus two standard deviation units from the mean of the corresponding control group was scored as a positive indicator of IBDV infection. Opened bursae were fixed by immersion in 10% neutral buffered formalin. Transverse portions of bursae were processed through graded alcohols and xylene, embedded into paraffin, sectioned, stained with hematoxylin-eosin, and examined with a light microscope. Protection against challenge was defined as the absence of any IBDV-induced lesions in the bursa of Fabricius.

#### **Serological evaluation**

The Classic D78 strain, as well as the cell culture adapted variant GLS strain of IBDV were grown in primary chicken embryo fibroblast cells and used in virus neutralization (VN) tests to test sera from the vaccine trial essentially as described (Snyder et al (1988a) "Avian Dis.", 32:527-534). Serum from the trials was also tested for the presence of anti-IBDV antibody using a commercially available IBDV antibody ELISA kit (Kirkegaard and Perry, Gaithersburg, MD).

#### **Evaluation of vaccines and challenge viruses**

The antigenic content of the I-7 GLS chimeric IBDV vaccine was assessed in AC-ELISA with a panel of VP2 and VP3 specific Mabs. The relative antigenic mass of each epitope expressed in the I-7 vaccine was compared to previously tested lots of *Baculovirus* expressed unmodified GLS subunit vaccines (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). The status of each Mab defined epitope on the I-7 chimeric vaccine was also compared to the status of those Mab defined epitopes

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occurring on wild type IBDV challenge viruses used to evaluate the efficacy of the I-7 vaccine. Table 3 shows that antigenic mass levels at the 8, 57, and B29 epitopes for the current I-7 chimeric vaccine were similar to a recently tested unmodified V-IBDV-7-1 GLS subunit vaccine, but approximately 4-fold higher than the original unmodified V-IBDV-7 vaccine.

TABLE 3

Comparative levels of IBDV, VP2, and VP3 monoclonal antibody (Mab) defined epitopes of recombinant baculovirus expressing IBDV proteins and status of Mab defined epitopes on challenge viruses used.

Vaccine	Relative level of Mab epitope <sup>A</sup>				Challenge	Status of Mab epitope <sup>B</sup>					
	8	57	B69	67		Virus	8 <sup>C</sup>	57 <sup>C</sup>	B69 <sup>C</sup>	67 <sup>C</sup>	
V-IBD-7 <sup>E</sup>	1	1	0	0	1	GLS	+	+	-	-	+
V-IBD-7-1 <sup>E</sup>	3	3	0	0	2	STC	+	-	+	-	+
I-7 <sup>F</sup>	3	3	9	0	2	IM	+	-	+	-	+
					E/DeI		+	-	-	+	+

<sup>A</sup> The relative level of each Mab epitope was determined by AC-ELISA, and the level of each Mab epitope was set to 1 for the V-IBD-7 vaccine previously used (15). Maximum level is 9. Each 1.0 increment represents approximately twice the amount of the epitope present in the original V-IBD-7 vaccine. The V-IBD-7-1 vaccine was also previously reported (16).

<sup>B</sup> The status of Mab epitopes was determined by AC-ELISA and is presented as present (+) or absent (-).

<sup>C</sup> Neutralizing Mab epitope resides on VP2 of IBDV.

<sup>D</sup> Non-neutralizing Mab epitope resides on VP3 or IBDV.

<sup>E</sup> Recombinant baculovirus vaccines incorporating unmodified large segment A GLS proteins.

<sup>F</sup> Current recombinant baculovirus vaccine incorporating modified chimeric large segment A GLS proteins.

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A major difference in the unmodified and chimeric vaccines was the appearance of the classic B69 epitope in the chimeric GLS product. The level of the B69 epitope was arbitrarily set at 9 since no comparisons could be made to the unmodified GLS subunit vaccines. By comparing the status of Mab defined epitopes on the challenge viruses with the unmodified and chimeric GLS subunit vaccines (Table 3), it could be seen that while the chimeric product had expressed the B69 epitope found on the Classic STC and IM challenge viruses, that it also retained all of the homologous GLS epitopes.

**Active-cross protection**

Table 4 shows the results of a cross-protection trial and serological results obtained prior to challenge.

TABLE 4  
Active cross-protection induced 2-weeks post immunization with baculovirus expressed chimeric I-7 IBDV antigens and associated prechallenge serology.

Group No.	Vaccination <sup>a</sup>	Challenge <sup>b</sup>	Number Protected			Mean VN Titer <sup>c</sup> Log		Mean ELISA
			AC-ELISA <sup>c</sup>	Histo <sup>d</sup>	BBWR <sup>d</sup>	D78	GLS	
I	None	None	N/A	NA	NA	≤4	≤4	0
II	None	STC	0/5	0/10	0/10	≤4	≤4	0
III	None	IM	0/5	0/5 <sup>b</sup>	5/5 <sup>b</sup>	≤4	≤4	0
IV	None	E/De1	0/5	0/10	0/10	≤4	≤4	0
V	None	GLS-5	0/5	0/10	0/10	≤4	≤4	0
VI	I-7	STC	5/5	10/10	10/10	107.7(1.8) <sup>e</sup>	10.4(1.4) <sup>e</sup>	1235(3112) <sup>f</sup>
VII	I-7	IM	5/5	10/10	10/10	10.0(1.4)	10.4(2.1)	1201(791)
VIII	I-7	E/De1	5/5	10/10	10/10	11.4(1.2)	10.6(1.9)	1089(409)
IX	I-7	GLS-5	5/5	10/10	10/10	11.0(1.5)	12.0(2.0)	1220(339)
X	I-7	None	5/5	NA	NA	9.9(1.4)	9.3(1.4)	1140(473)

<sup>a</sup>Vaccination was given at 8-weeks of age.

<sup>b</sup>Challenge virus was given by intraocular instillation 3-weeks post immunization or at 11-weeks of age for controls.

<sup>c</sup>Protection was determined by AC-ELISA examination of 1/3 of each group 4-days post-challenge.

<sup>d</sup>Protection was determined histologically and by bursa to body weight ratios at 8-days.

<sup>e</sup>Five chickens were scored as dead due to IM challenge prior to 8-days post-challenge.

<sup>f</sup>One standard deviation.

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Groups II - V served as challenge controls and as indicated by AC-ELISA, bursa to body weight and histological assessments, all non-vaccinated chickens were fully susceptible to virulent IBDV challenge with all strains used. The IM challenge produced lethal disease in one-third of the control group chicks. In contrast, 8-week old chickens comprising Groups VI - IX were vaccinated once with the GLS chimeric vaccine, and 3-weeks PI all vaccinated chickens were completely protected from challenge by all challenge viruses, including lethal disease produced in controls by the IM strain. Serologically, titers from reciprocal-cross VN tests conducted on prechallenge sera with the D78 and GLS tissue culture viruses were essentially within 2-fold of one another. Mean ELISA titers were relatively low, but were also uniform between the vaccinated groups.

#### Characterization of vaccines

In initial studies with *Baculovirus* expressed subunit GLS vaccines, after administration of two doses, the V-IBDV-7 GLS vaccine (Table 3) could only induce active antibody levels capable of providing 79% protection against homologous GLS challenge (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In a subsequent study, the antigenic mass of the original V-IBDV-7 vaccine was increased approximately 4-fold (calculated at the group specific Mab 8 site) and initiated one dose and two dose vaccination cross-challenge trials with the unmcdified GLS subunit vaccine designated as V-IBDV-7-1 (Table 3). In those trials, two doses of the vaccine yielded complete cross-protection against virulent STC, E/DEL and GLS challenge. However, in the one vaccine dose trial, while complete protection was attained against challenge with variant E/DEL and GLS viruses, only 44% protection was achieved against the more distantly related Classic STC virus. Those studies could mean that simply by increasing the antigenic mass and/or doses of the vaccine that better cross-protection could be obtained. However, it was also evident in the absence of homologous vaccination that lower levels of

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antibody, induced by one dose of the GLS V-1BDV-7-1 subunit vaccine, were not sufficiently cross-protective against Classic IBDV challenge. This could mean that in even lower levels of antibody, such as in cases of waning maternal antibody, that cross-protection would likely be even more reduced. Indeed, although not challenged with the STC virus, in some passive maternal antibody studies conducted using another dosage of the V-1BDV-7 vaccine, while homologous GLS protection was afforded, progeny of vaccinated hens were only 57% protected against a more closely related E/DEL challenge.

In a single-dose vaccination cross-challenge trial, the chimeric GLS I-7 vaccine, which incorporated the Classic B69 neutralization epitope, was evaluated. In order to make the current trial comparable to previous trials, the I-7 vaccine was formulated such that by AC-ELISA the relative antigenic mass of the I-7 chimeric subunit vaccine was nearly identical to the unmodified V-IBDV-7-1 vaccine previously used (Table 3). Table 4 shows the results of the cross-challenge after a single dose of the I-7 vaccine was administered. Results were similar to those obtained with the unmodified V-IBDV-7-1 vaccine previously used in that protection against the GLS and E/DEL strains was complete. However, the I-7 vaccine yielded complete protection against pathogenic and lethal challenge by the Classic STC and IM strains respectively. Since the antigenic mass of the GLS and group common epitopes on V-IBDV-7 and I-7 vaccines were carefully equilibrated and equal, it is reasonable to conclude that the comparative increase in efficacy of the I-7 vaccine against challenge with Classic IBDV strains was due solely to the incorporation of the Classic IBDV B69 neutralization epitope in the GLS VP2 protein sequence.

#### VIRUS-LIKE PARTICLES

As noted above, the recombinant cDNA and immunogens expressed thereby, of this invention may be confined to the VP2 immunogenic region. In other words, it may be sufficient to prepare a cDNA clone encoding epitopic determinants for a

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base IBDV, e.g., GLS, as well as a second IBDV epitopic determinant, such as D78. Other epitopic determinants, all in the VP2 epitopic determinant region may be incorporated, cloned and expressed as discussed above.

As reflected in Figure 2, virus-like particles are generated by the expression of DNA encoding the VP2-VP4-VP3 structural protein sequences. These virus-like particle immunogens can be separated from the corresponding VP2 only immunogens, both in terms of monoclonal antibody and by conventional separation measures, such as electrophoresis and chromatography. The difference in reactivity with monoclonal antibody strongly indicates, however, that epitopes present in the VP2-VP4-VP3 structural protein sequence-induced virus-like particles are present that are not present in immunogens expressed by the identical VP2 only region. These epitopes are "both linear and conformational" epitopes. Conformational epitopes differ from linear epitopes and are reflected in the conformation, not only in amino acid sequence of the actual virus. As a result, inoculation of poultry with a recombinant virus-like particle may provide even superior protection against field challenge from IBDV than inoculation with the immunogens of the VP2 region only. This is due to the spontaneous assembly of all the structural elements of the virus.

Applicants have discovered that the expression of the VP2 region as part of the VP2-VP4-VP3 structural protein single segment generates virus-like particles such as those of Figure 2. These particles have been demonstrated to react with antibodies which do not react similarly with the identical recombinant VP2 immunogen. Thus, the virus-like particles may give rise to higher antibody titers, and/or subtly different (broader) protection when a poultry host is inoculated therewith.

The invention herein therefore embraces (1) recombinant VP2 immunogens comprising epitopic determinants of at least two different IBDV strains and (2) virus-like particles of VP2-VP4-VP3 segments wherein the VP2 region again comprises

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epitopic determinants of at least two different IBDV strain, as well as the nucleotide sequences encoding both 1 and 2, and vaccines embracing the same.

RECOMBINANT EPITOPIIC DETERMINANT COMBINATIONS:

As reflected in the examples set forth above, genetic epitopic determinants for an IBDV strain can be inserted in the VP2 region of a different, base IBDV genetic sequence, and subsequently used to express an immunogen exhibiting epitopes for both IBDV. Indeed, the examples above demonstrate the combination of at least three different IBDV epitopic determinants. More can be combined. The resulting vaccine includes an active agent, the expressed immunogen, which provides challenge protection against a broad spectrum of IBDV, rather than prior art virus-based vaccines which give protection against a single strain, or a single family of strains.

Figure 7 reflects the amino acid identities for the epitopic determinant region for seven different IBDV. These are not intended to be limiting, but are representative. Desirable recombinant immunogens, both VP2 only and virus-like particle VP2-VP4-VP3 immunogens are made by substituting the genetic epitopic determinants for the varying amino acids at the identified locations in Figure 7 (locations not identified are conserved throughout the IBDV strains). This induces the expression of the inventive immunogens. Clearly, the possible combinations, while large in number, are limited, and may be investigated with routine skill. Representative combinations will tend to reflect combinations of epitopic determinants for dominant IBDV.

A E/Del/GLS recombinant may include changes in the E/Del epitopic determinant region at position 213, Asn-Asp, 253 Gln-His and 169 Thr Ser.

A DS326/D78 recombinant may include the amino acid, and corresponding nucleotide substitutions at 76Ser-Gly, 249 Lys-Gln, 253 Gln-His and 270 Ala-Thr substitutions.

Obviously, a wide variety of combinations are possible

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and will occur to those of skill in the art. The epitopic determinant region, roughly including the region from amino acid 5-433 of the VP2 region, thus constitutes a recombinant "cassette" which may be tailored by site-specific mutagenesis to achieve amino acid insertion and/or deletion to provide desired recombinant cDNA clones, polypeptides, virus-like particles and vaccines with improved protection against a wide variety of IBDV.

LETHAL IBDV, MONOCLONAL ANTIBODY AND VACCINE THEREFORE

As noted, typically, IBDV infection creates an immunosuppressive condition, and is reflected in lesions in the bursa of Fabricius. This is typical of IBDV countered in the United States. There exist, however, lethal IBDV, that is, IBDV infections which results in chicken mortality directly as a result of IBDV infection. While vaccines have been developed on the basis of isolation of these IBDV, the resulting vaccines are "hot", that is, they themselves create or induce an immunosuppressive condition, and the inoculated chick must be bolstered with antibodies to other infectious agents. This method of protection is so undesirable as to have been discontinued in most commercial poultry houses in Europe. No adequate safe vaccine against the lethal IBDV is currently available.

The inventors have developed a monoclonal antibody, Mab 21, deposited under Budapest Treaty conditions at the American Type Culture Collection, Deposit Accession No. ATCC HB 11566. This monoclonal antibody is specific and neutralizing for lethal IBDV strains. The specificity is reflected in Table 5, which confirms that unlike other monoclonal antibody, Mab 21 is specific for an epitope exhibited only by IBDV strains having lethal potential.

TABLE 5

Source	IBDV Strain		Comment	829	8	179	10	63	21	67	57	50	
	Lethal Potential	TM+											
Sharma													
USDA	IM	STC	2512 (Winterfield)										
Spates				(vaccine hot)									
Edgar													
			Pathogenic Virus										
Sterwin			Bursa Vac										
			Vaccine Virus										
ASL			Univax-BD	(ST 14)									
Select			Bursal Disease Vaccine	(Luk)									
Select				(STD + VAR)									
Key Vet				(D78)									
Key Vet			Bio-Burs 1	(Luk)									
Key Vet			Bio-Burs 4	(D78)									
MBL			Key-Burs	(Master seed)									
Sterwin			Maryland	(Basendale									
Sterwin			BVM	BVM									
Lukert			1048-E										
CEVA			BVM	(Lab Strain)									
Intervet			Bursa Blend	(2512)									
Intervet			D78										
Intervet			Prime Vac										
Solvay			8903										
Solvay			Bursine										
Solvay			Bursine 11										
			Lab Virus										
JKR			E/Del										
JKR			A/Del										
KKR			D/Del										
DBS			GLS										
DBS			DS26										
*Skeels			\$977	(Serotype 11)									
OH													

\* Field strains: All classic field strains tested to date which were isolated in the U.S. have the 21 marker. NOTE: 1. Lukert and STC are Edgar derivatives. 2. Univax is a Bursa Vac derivative. 3. Bursa Blend is a 2512 Winterfield derivative.

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It should be noted that throughout this application, reference is made to a variety of monoclonal antibody which are used to confirm the presence of epitopes of different IBDV in the inventive recombinant chimeric immunogens of the application. These monoclonal antibody have also been deposited under Budapest Treaty conditions and are freely available. They are not, however, necessary for the practice of this invention, and do not constitute an aspect thereof. This should be contrasted with Mab 21.

Like other Mab developed by the inventors herein for IBDV, passive immunization against IBDV lethal strains, particularly designed to achieve immunization in a uniform, standardized level, and to augment any maternally derived levels against lethal IBDV field infection can be obtained by vaccinating one-day old chicks with a vaccine comprising a pharmacologically acceptable carrier such as those described above, in which is present an amount of Mab 21 effective to provide enhanced protection for the inoculated chicks.

The necessary level of protection can be conferred to by a single dose of the vaccine administered in ova or to a day-old chick having a Mab 21 concentration of between 1 microgram and 1 milligram, or repeated vaccinations having a smaller effective dose, but carried out over time. If repeated vaccinations are used, the dosage levels should range between 1 microgram and 1 milligram. The concentration level needed to vaccinate older chickens increases with the weight of the bird and can be determined empirically.

Further investigation of the amino acid sequences of the lethal strains in the epitopic determinant region reflects the highly conserved 279 identity Asn at position 279 of VP2, in non-lethal strains, with a conserved Asp identity at the same position in lethal strains. Accordingly, the lethal strain epitopic determinant recognized by Mab 21, unique to the lethal strains, empirically differs from non-lethal IBDV by the substitution 279 Asp-Asn. According to the methods set forth above, a chimeric, recombinant immunogen conferring effective protection against lethal IBDV, something not

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possible previously with any type of vaccine without inducing an immunosuppressive condition, may be prepared by inserting the genetic epitopic determinant for 279 Asp in a non-lethal base IBDV, such as GLS. This will confer protection against the base IBDV, the lethal IBDV, as well as all other IBDV whose genetic epitopic determinants are inserted. Vaccines prepared from these immunogens, whether VP2 only, or in the form of virus-like particles of VP2-VP-VP3 segments, are used in the same fashion as discussed above.

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Claims:

1. A chimeric polypeptide immunogen comprising the VP2 amino acid sequence of a first infectious bursal disease virus (IBDV) except for at least one amino acid X, wherein X is an epitopic determinant from a second IBDV strain.
2. The immunogen of Claim 1, wherein said VP2 amino acid sequence comprises a plurality of a different epitopic determinant X.
3. The immunogen of Claim 2, wherein said plurality of epitopic determinants X are from at least two different IBDV strains.
4. The immunogen of Claim 1, wherein said IBDV strains are selected from the group consisting of GLS, E/Del, D78, DS326, RS593, Cu-1, PBG98, 52/70, STC and 002-73.
5. The immunogen of Claim 1, wherein said immunogen comprises the amino acid sequence, in order, for IBDV structural proteins VP2-VP4-VP3.
6. The immunogen of Claim 5, wherein said immunogen is in the form of a virus-like particle.
7. The immunogen of Claim 6, wherein said immunogen exhibits at least one IBDV conformational epitope.
8. The immunogen of Claim 1, wherein said amino acid sequence includes an epitopic determinant X of a lethal IBDV strain.
9. The immunogen of Claim 8, wherein said epitopic determinant of lethal IBDV strains comprises the amino acid Asp at position 279 of the VP2 sequence.

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10. A preparation sufficient to provide poultry inoculated therewith resistance to IBDV challenge from at least two different IBDV strains, comprising, as an active agent, an effective amount of the immunogen of any one of Claims 1-9, and a pharmacologically acceptable carrier.

11. An avirulent immunogen which confirms on poultry inoculated therewith protection against challenge from IBDV lethal strains, said immunogen comprising the VP2 amino acid sequence of an IBDV, wherein position 279 of said VP2 amino acid is Asp.

12. The immunogen of Claim 11, wherein said immunogen comprises, in order, amino acid sequences for VP2-VP4-VP3 IBDV structural proteins.

13. The immunogen of Claim 12, in the form of virus-like particles.

14. A monoclonal antibody which binds, under AC-ELISA conditions, to IBDV lethal strains, and has the epitope binding characteristics of the monoclonal antibody expressed by the cell line deposited under Accession No. ATCC HB 11566.

15. The monoclonal antibody of Claim 14, wherein said monoclonal antibody is obtained, directly or indirectly, from said cell line.

16. The monoclonal antibody of Claim 15, wherein said antibody is the antibody expressed by said cell line.

17. A preparation for conferring passive immunity in a poultry inoculated therewith against IBDV lethal strain challenge, comprising, as an effective agent, the monoclonal antibody of any one of Claims 14-16 in an effective amount, and

a physiologically acceptable carrier.

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18. A chimeric cDNA which, when operably inserted as heterologous DNA in the DNA of an expression host, encodes the immunogen of any one of Claims 1-9.

19. A transfection vehicle for the infection of an expression host, comprising the cDNA of Claim 18 as operably connected in the DNA of *baculovirus* *fowlpox virus*, *turkey herpes virus* or *adenovirus*.

20. An expression vehicle for the expression of the immunogen of Claims 1-9, comprising an expression host selected from the group consisting of SF9 cells, chicken embryo fibroblast cells, chicken embryo kidney cells and vero cells transfected with the vehicle of Claim 19.

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5' | 1kb | 2kb | 3kb | 3'



pGLS-5



pB69GLS

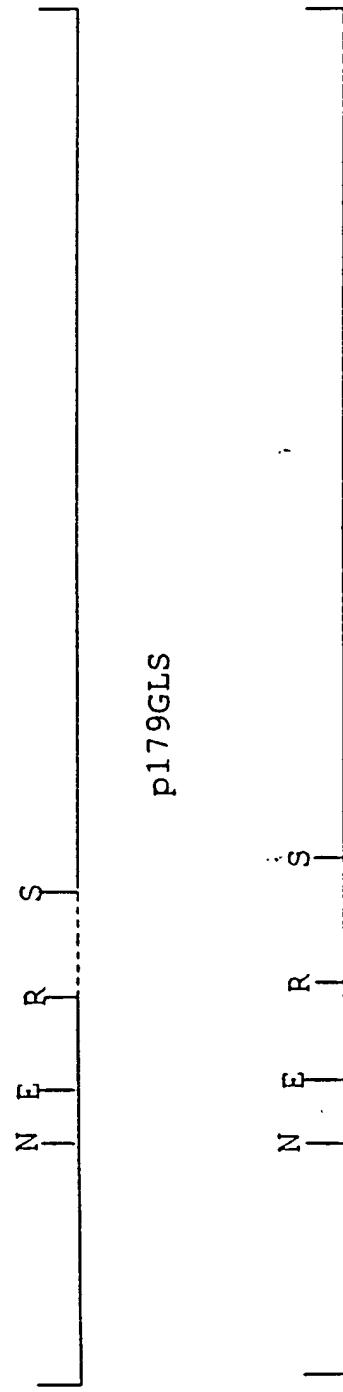


pR63GLS

FIG. 1A

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FIG. 1B



pE/De1-22

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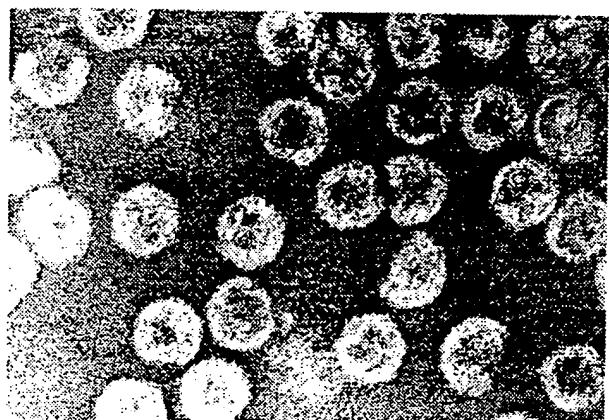


FIG. 2A

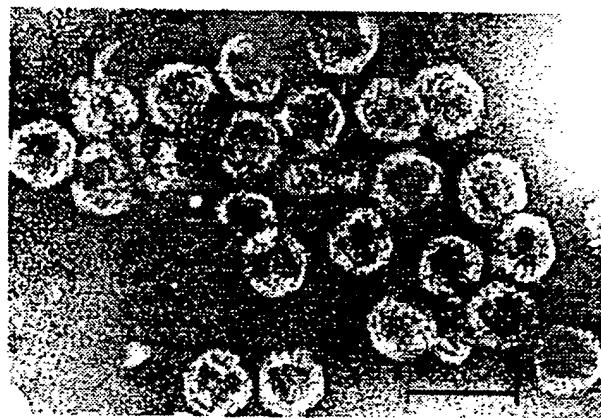


FIG. 2B

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FIG. 3A

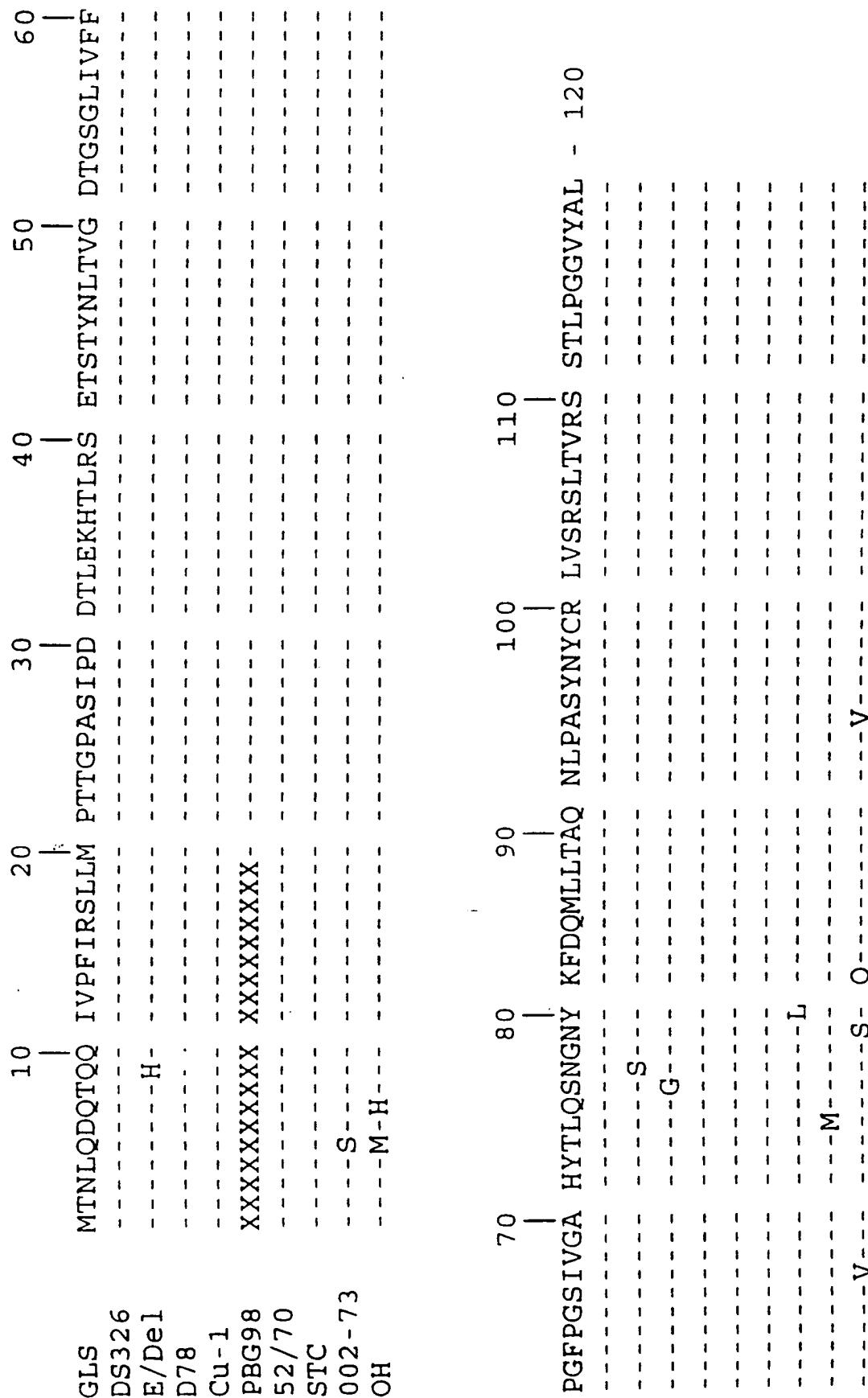


FIG. 3B

GLS	NGTINAVTFO	GSLSELTDVS	YNGLMSATAN	INDKIGNVLV	GEGVTVLSLP	TSYDLGYVRL
DS326						
E/De1						
D78						
Cu-1						
PBG98						
52/70						
STC						
002-73						
OH						

FIG. 3C

10 | S V G G E L V F K T S V H S L V L G A T I Y L I G F D G S A V I T R A V A A N N G L T T G T D N I L M P F N L V I P T N E  
 20 | - - - - - Q - - - - - T - - - - - A - - - - -  
 30 | - - - - - Q - - - - - T - - - - - A - - - - -  
 40 | - - - - - Q - - - - - T - - - - - A - - - - -  
 50 | - - - - - Q - - - - - T - - - - - A - - - - -  
 60 | - - - - - Q - - - - - T - - - - - A - - - - -  
 GLS S V G G E L V F K T S V H S L V L G A T I Y L I G F D G S A V I T R A V A A N N G L T T G T D N I L M P F N L V I P T N E  
 DS326 - - - - - Q - - - - - T - - - - - A - - - - -  
 E/De1 - - - - - Q - - - - - T - - - - - A - - - - -  
 D78 - - - - - Q - - - - - T - - - - - A - - - - -  
 Cu-1 - - - - - Q - - - - - T - - - - - A - - - - -  
 PBG98 - - - - - R - - - - - T - - - - - A - - - - -  
 52/70 - - - - - Q - - - - - T - - - - - A - - - - -  
 STC - - - - - Q - - - - - T - - - - - A - - - - -  
 002-73 - - - - - Q - - - - - T - - - - - A - - - - -  
 OH - - - - - I - S Q V T I - S I E V D V - - F - - - T E - T V K - - T D F - - N - V - - G G - - S - - 6/38

70 | 80 | 90 | 100 | 110 |  
 I T Q P I T S I K L E I V T S K S G G Q E G D Q M S W S A S G S L A V T I H G G N Y P G A L R P V T L V A Y E R V A T G - 3 6 0  
 - - - - - K - - - - - L - - - - -  
 - - - - - I - - - - - D - - A - E - - - - -  
 - - - - - - - - - A - - - - - R - - - - -  
 - - - - - - - - - A - - - - - K - - - - -  
 - - - - - - - - - A - - - - - L - - R - - - - -  
 - - - - - - - - - A - - - - - - - - - - -  
 - - - - - V - - - - - A - - - - - - - - - - -  
 - - - - - V - - - - - A - - - - - L - - - - -  
 - - - - - M - - - - - V - - - - - V - - - - -  
 - - - - - V - - - - - A - - - - - P I - - T V - - - - - A - - - - - 3 6 1

## FIG. 3D

10 | SVVTVAGVSN FELIPNPELA KNLVTEYGRF DPGAMNYTKL ILSERDRLGI KTVWPTREYT  
 20 |  
 30 |  
 40 |  
 50 |  
 60 |  
 GLS  
 DS326  
 E/De1  
 D78  
 Cu-1  
 PBG98  
 52/70  
 STC  
 002-73  
 OH

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70 | DFREYEMEVA DLSSPLKIAG AFGFKDIIRA IRRIAVPVVS TLEPPAAPLA HAIGEGVDYL - 480  
 80 |  
 90 |  
 100 |  
 110 |  
 K  
 NR  
 - 481

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## FIG. 3E

10	20	30	40	50	60	
GLS	LGDEAQAAASG	TARAASGKAR	AASGRIRQLT	LAADKGYEVV	ANLFQVPQNP	VVDGILASP
DS326						
E/De1						
D78						
Cu-1						
PBG98						
52/70						
STC						
002-73						
OH			G		M	I

70	80	90	100	110		
ILRGAHNLD	VLREGATLFP	VVITTVEDAM	TPKALNSKMF	AVIEGVREDL	QPPSQRGSFI	- 600
V					A	
V						
V						
V						
V						
V					I	
V						
V						
V						
SK			EL		A	- 601

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FIG. 3F

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FIG. 3G

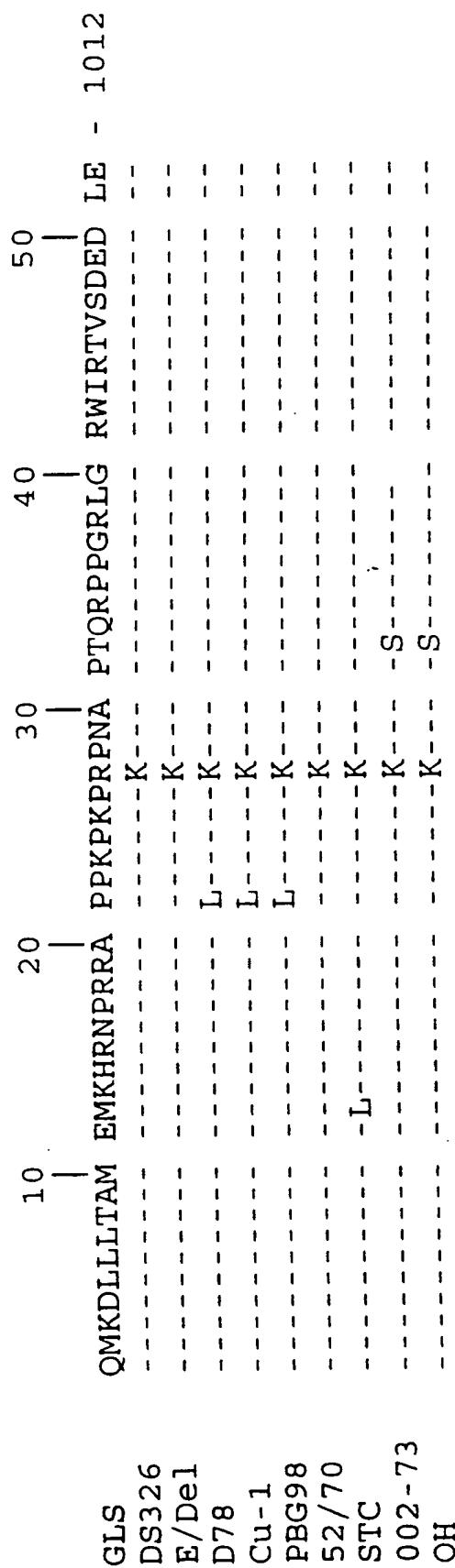
GLS	IKRFPHNPRD	WDRLLPYILNLP	YLPPNAGRQY	HLAMAASEEK	ETPELESAVR	AMEAAASVDP
DS326	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
E/De1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
D78	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Cu-1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
PBG98	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
52/70	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
STC	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
002-73	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
OH	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -

### FIG. 3H

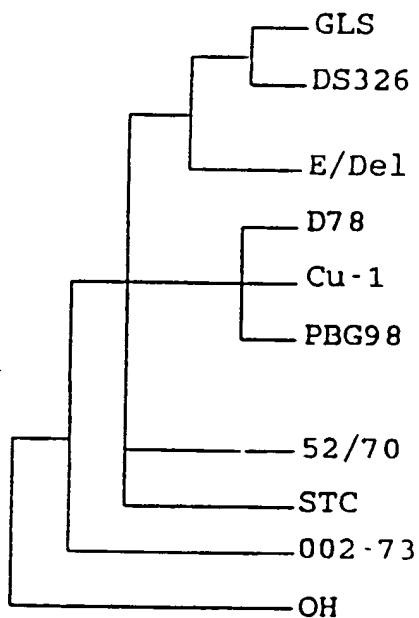
10	20	30	40	50	60				
EARGPTPEEA	QREKDTRISK	KMETMGIYFA	TPEWVALNGH	RGPSPGQLKY	WQNNTREIPDP				
GLS	DS326	E/De1	D78	Cu-1	PBG98	52/70	STC	002-73	OH

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FIG. 3I



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*FIG. 4*

\*\*\*\*\*  
 \* TRANSLATION OF A NUCLEIC ACID SEQUENCE \*  
 \*\*\*\*\*

### FIG. 5A

Done on large genome segment A of GLS-IBDV.

DE From cDNA clones pGLS-1 to pGLS-4.

Total number of bases is: 3230.

Analysis done on bases 114 to 3152.

Done on (absolute) phase(s): 3.

Using the Universal genetic code.

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60	70	80	90	100	110	120	130	140	150	160								
CTT	CTA	CAA	TGC	TAT	CAT	TGA	TGG	TTA	GTA	GAG	ATC	GAA	CAA	ACG	ATC	GCA	CCG	CCC
ATG	ACA	AAC	CTG	CAA	GAT	CAA	ACC	CAA	CAG	ATT	GTT	CCG	TTC	ATA	CGG	AGC	CTT	
MET	Thr	Asn	Leu	Gln	Asp	Gln	Thr	Gln	Ile	Val	Phe	Ile	Arg	Ser	Leu			

FIG. 5B

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## FIG. 5C

500	GTG ACC TTC CAA GGA AGC CTG AGT GAA CTG ACA GAT	GTT AGC TAC AAT GGG	TTC
Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gln Gly Leu			
510			
520			
530			
540			
550	ATG TCT GCA ACA GCC AAC ATC AAC GAC AAA ATT GGG	AAC GTC CTA GTA GGG	GAA
MET Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val Gln Glu			
560			
570			
580			
590			
600	GGG GTT ACT GTC CTC AGC TTA CCC ACA TCA TAT GAT	CTT GGG TAT GTG AGG	CTT
Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly Tyr Val Arg Leu			
610			
620			
630			
640			
650			
660	GGT GAC CCC ATA CCC GCT ATA GGG CTT GAC CCA AAA	ATG GTA GCA ACA TGT GAC	
Gly Asp Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys MET Val Ala Thr Cys Asp			
670			
680			
690			
700			
710	AGC AGT GAC AGG CCC AGA GTC TAC ACC ATA ACT GCA GCT GAT GAC	TAC CAA TTC	
Ser Ser Ser Asp Asp Arg Pro Arg Val Tyr Thr Ile Thr Ala Ala Asp Asp Tyr Gln Phe			
720			
730			
740			
750			
760			
770	780	790	800
TCA TCA CAG TAC CAA ACA GCA GGT GGG GTA ACA ATC ACC CTG RTC TCA GCC AAC ATT			
Ser Ser Gln Tyr Gln Thr Gln Thr Gly Gly Val Thr Ile Thr Leu Phe Ser Ala Asn Ile			
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## FIG. 5D

820	830	840	850	860																		
GAT	GCC	ATC	ACA	AGC	CTC	AGC	GTC	GGG	GAG	CTC	GTG	TTT	AAA	ACA	AGC	GTC						
Asp	Ala	Ile	Thr	Ser	Leu	Ser	Val	Gly	Gly	Gly	Leu	Val	Phe	Lys	Thr	Ser	Val					
870	880	890	900	910	920																	
CAC	AGC	CTT	GTA	CTG	GGC	GCC	ACC	ATC	TAC	CTT	ATA	GGC	TTT	GAT	GGG	TCT	GCG					
His	Ser	Leu	Val	Leu	Gly	Ala	Thr	Ile	Tyr	Leu	Ile	Gly	Phe	Asp	Gly	Ser	Ala					
930	940	950	960	970	980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100	1110	1120	1130		
GTA	ATC	ACT	AGA	GCT	GTG	GCC	GCA	AAC	AAT	GGG	CTG	ACG	ACC	GGC	ACC	GAC	AAT	Val	Ile	Thr	Asn	
Val	Ile	Thr	Arg	Ala	Val	Ala	Ala	Asn	Asn	Gly	Leu	Thr	Thr	Gly	Thr	Asp	Asn					
CCT	ATG	CCA	TTC	AAT	CTT	GTG	ATT	CCA	ACC	AAC	GAG	ATA	ACC	CAG	CCA	ATC	ACA	Leu	MET	Pro	Phe	Asn
Ser	Ile	Lys	Leu	Ile	Val	Leu	Val	Pro	Thr	Asn	Glu	Ile	Thr	Gln	Pro	Ile	Thr					

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FIG. 5E

1140	1150	1160	1170	1180
CCA	GGG	GCC	CTC	CGT
Pro	Gly	Ala	Leu	Arg
1200	1210	1220	1230	1240
TCT	GTC	GTT	ACG	GTC
Ser	Val	Val	Thr	Val
1250	1260	1270	1280	1290
CTA	GCA	AAG	AAC	CTG
Leu	Ala	Lys	Asn	Leu
1310	1320	1330	1340	1350
TAC	ACA	AAA	TTG	ATA
Tyr	Thr	Lys	Leu	Ile
1360	1370	1380	1390	1400
CCG	ACA	AGG	GAG	TAC
Pro	Thr	Arg	Glu	Tyr
1410	1420	1430	1440	1450
AGC	TCT	CCC	AAG	ATT
Ser	Ser	Pro	Leu	Leu

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**FIG. 5F**

1470	1480	1490	1500	1510
ATA AGG ATA GCT GTTG CCG GTG GTC TCC ACA TTC CCT GGC CCC				
Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro Pro Ala Ala Pro				
1520	1530	1540	1550	1560
CTG GCC CAT GCA ATT GGG GAA GGT GTC GAC TAC CTG CTG GGT GAT GAG GCA CAG				
Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu Leu G1y Asp Glu Ala Gln				
1580	1590	1600	1610	1620
GCT GCT TCA GGA ACT GCT CGA GCC GCG TCA GGA AAA GCA AGG GCT GCC TCA GGC				
Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser Gly Lys Ala Arg Ala Ala Ser Gly				
1630	1640	1650	1660	1670
CGC ATA AGG CAG CTG ACT CTC GCC GCC GAC AAG GGG TAC GAG GTC GCG AAT				
Arg Ile Arg Gln Leu Thr Leu Ala Asp Lys Gly Tyr Glu Val Val Ala Asn				
1680	1690	1700	1710	1720
CTA TTC CAG GTG CCC CAG AAT CCC GTA GTC GAC GGG ATT CTT GCT TCA CCC GGG				
Leu Phe Gln Val Pro Gln Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly				
1740	1750	1760	1770	1780
ATA CTC CGC GGT GCA CAC AAC CTC GAC TGC GTG TTA AGA GAG GCC GCC ACG CTA				
Ile Leu Arg Gly Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu				

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**FIG. 5G**

1790	1800	1810	1820	1830	1840
TTG CCT GTC GTC ATC ACG ACA GTC GAA GAC GCC ATG ACA CCC AAA GCA CTA AAC					
Phe Pro Val Val Ile Thr Thr Val Glu Asp Ala MET Thr Pro Lys Ala Leu Asn					
1850	1860	1870	1880	1890	1900
AGC AAA ATG TTT GCT GTC ATT GAA GGC GTG CGA GAG GAC CTC CAA CCT CCA TCT					
Ser Lys MET Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro Pro Ser					
1900	1910	1920	1930	1940	1950
CAA AGA GGA TCC TTC ATA CGA ACT CTC TCC GGA CAC AGA GTC TAT GGA TAT GCT					
Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val Tyr Gly Tyr Ala					
1960	1970	1980	1990	2000	2010
CCA GAT GGG GTC CTT CCA CTG GAG ACT GGG AGA GAC TAC ACC GTT GTC CCA ATA					
Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp Tyr Thr Val Val Pro Ile					
2020	2030	2040	2050	2060	2070
GAT GAT TGG GAC GAC AGC ATT ATG CTG TCC AAA GAC CCC ATA CCT CCT ATT					
Asp Asp Val Trp Asp Asp Ser Ile MET Leu Ser Lys Asp Pro Ile Pro Pro Ile					
2080	2090	2100	2110		
GTG GGA AAC AGT GGA AAC CTA GCC ATA GCT TAC ATG GAT GTG TTT CGA CCC AAA					
Val Gly Asn Ser Gly Asn Leu Ala Ile Ala Tyr MET Asp Val Phe Arg Pro Lys					

FIG. 5H

2120	CCC	ATC	CAT	GTG	GCC	ATG	ACG	GGA	GCC	CTC	AAC	GCT	TGT	GGC	GAG	ATT	GAG	
GTC	CCC	ATC	CAT	GTG	GCC	ATG	ACG	GGA	GCC	CTC	AAC	GCT	TGT	GGC	GAG	ATT	GAG	
Val	Ile	His	Val	Ala	MET	Thr	Gly	Ala	Leu	Asn	Ala	Cys	Gly	Glu	Ile	Glu		
2170																		2160
AAA	ATA	AGC	TTT	AGA	AGC	ACC	AAG	CAC	ACC	GCC	ACC	GCA	CAC	CGG	CTT	GGC	CAC	
Lys	Ile	Ser	Phe	Arg	Ser	Thr	Lys	Leu	Ala	Thr	Ala	His	Arg	Leu	Gly	Leu	Lys	
2220																		2150
TTG	GCT	GGT	CCC	GGA	GCA	TTT	GAT	GTA	AAC	ACC	GGG	CCC	AAC	TGG	GCA	ACG	TCG	
Leu	Ala	Gly	Pro	Gly	Ala	Phe	Asp	Val	Asn	Thr	Gly	Pro	Asn	Trp	Ala	Thr	Phe	
2280																		2210
ATC	AAA	CGT	TTC	CCT	CAC	AAT	CCA	CGC	GAC	TGG	GAC	AGG	CTC	CCC	TTC	CTC	AAC	
Ile	Lys	Arg	Phe	Pro	His	Asn	Pro	Arg	Asp	Trp	Asp	Arg	Leu	Pro	Tyr	Leu	Asn	
2330																		2140
CTT	CCA	TAC	CTT	CCA	CCC	AAT	GCA	GGA	CGC	CAG	TAC	CAC	CTC	GCC	ATG	GCC	GCA	
Leu	Pro	Tyr	Leu	Pro	Pro	Asn	Ala	Gly	Arg	Gln	Tyr	His	Leu	Ala	MET	Ala	Ala	
2390																		2160
TCA	GAG	TTC	AAG	GAG	ACC	CCT	GAA	CTC	GAG	AGC	GCC	GTC	AGG	GCC	GTC	ATG	GAA	GCA
Ser	Glu	Phe	Lys	Glu	Thr	Pro	Glu	Leu	Glu	Ser	Ala	Val	Arg	Ala	Val	Arg	Ala	Glu

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## FIG. 5I

2440	GCA	GCC	AGT	GTA	GAC	CCA	CTG	TTC	CAA	TCT	GCA	CTC	AGT	GTG	TTC	ATG	TGG	CTG
Ala	Ala	Ser	Val	Asp	Pro	Leu	Phe	Gln	Ser	Ala	Leu	Ser	Val	Phe	MET	Trp	Leu	
245	2460	2470	2480															
2490	GAA	GAG	AAT	GGG	ATT	GTG	ACT	GAC	ATG	GCC	AAC	TTC	GCA	CTC	AGC	GAC	CCG	AAC
Glu	Glu	Glu	Asn	Gly	Ile	Val	Thr	Asp	MET	Ala	Asn	Ala	Phe	Ala	Leu	Ser	Asp	Pro
2500	2510	2520	2530	2540														
2550	GCC	CAT	CGG	ATG	CGA	AAC	TTT	CTT	GCA	AAC	GCA	CCA	GCA	GGT	AGC	AAG	TCT	
Ala	His	Arg	MET	Arg	Asn	Phe	Leu	Ala	Asn	Ala	Pro	Gln	Ala	Gly	Ser	Lys	Ser	
2560	2570	2580	2590															
2600	2610	2620	2630	2640	2650													
CAA	AGG	GCC	AAA	TAC	GGG	ACA	GCA	GGC	TAC	GGG	GTG	GAG	GCC	GGG	CCC	ACA		
Gln	Arg	Ala	Lys	Tyr	Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	Arg	Gly	Pro	Thr	
2660	2670	2680	2690	2700														
CCA	GAA	GCA	CAG	AGG	GAA	AAA	GAC	ACA	CGG	ATC	TCA	AAG	AAG	ATG	GAG	ACC		
Pro	Glu	Glu	Ala	Gln	Arg	Glu	Lys	Asp	Thr	Arg	Ile	Ser	Lys	MET	Glu	Thr		
2710	2720	2730	2740	2750														
ATG	GGC	ATC	TAC	TTT	GCA	ACA	CCA	GAA	TGG	GTA	GCA	CTC	AAT	GGG	CAC	CGA	GGG	
MET	Gly	Ile	Tyr	Phe	Ala	Thr	Pro	Glu	Trp	Val	Ala	Leu	Asn	Gly	His	Arg	Gly	

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## FIG. 5J

2760	2770	2780	2790	2800	2810
CCA AGC CCC GGC CAG CTA AAG TAC TGG CAG AAC ACA CGA GAA ATA CCG GAC CCA					
Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu Ile Pro Asp Pro					
2820	2830	2840	2850	2860	
AAC GAG GAC TAT CTA GAC TAC GTG CAT GCA GAG AAG CGG AGC CCG TTG GCA TCA GAA					
Asn Glu Asp Tyr Leu Asp Asp Tyr Val His Ala Glu Lys Ser Arg Leu Ala Ser Glu					
2870	2880	2890	2900	2910	2920
GAA CAA ATC CTA AGG GCA GCT ACG TCG ATC TAC GGG GCT CCA GGA CAG GCA GAG					
Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile Tyr Gly Ala Pro Gly Gln Ala Glu					
2930	2940	2950	2960	2970	
CCA CCC CAA GCT TTC ATA GAC GAA GTT GCC AAA GTC TAT GAA ATC AAC CAT GGA					
Pro Pro Gln Ala Phe Ile Asp Glu Val Ala Lys Val Tyr Glu Ile Asn His Gly					
2980	2990	3000	3010	3020	
CGT GGC CCA AAC CAA GAA CAG ATG AAA GAT CTG CTC TTG ACT GCG ATG GAG ATG					
Arg Gly Pro Asn Gln Glu Gln MET Iys Asp Leu Leu Thr Ala MET Glu MET					
3030	3040	3050	3060	3070	3080
AAG CAT CGC AAT CCC AGG CGG GCT CCA CAG CCC AAG CCA AGA CCC AAC GCT					
Lys His Arg Asn Pro Arg Arg Ala Pro Pro Lys Pro Lys Pro Arg Pro Arg Pro Asn Ala					

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## FIG. 5K

3090	3100	3110	3120	3130																
CCA	ACG	CAG	AGA	CCC	CCT	GGT	CGG	CTG	GGC	CGC	TGG	ATC	AGG	ACT	GTC	TCT	GAT			
Pro	Thr	Gln	Arg	Pro	Pro	Gly	Arg	Leu	Gly	Arg	Leu	Gly	Arg	Trp	Ile	Arg	Thr	Val	Ser	Asp
3140	3150	3160	3170	3180	3190															
GAG	GAC	CTT	GAG	TGA	GGC	TCC	TGG	GAG	TCT	CCC	GAC	ACC	ACC	CGC	GCA	GGC	GTG			
Glu	Asp	Leu	Glu	--																
3200	3210	3220	3230																	
GAC	ACC	AAT	TCG	GCC	TTA	CAA	CAT	CCC	AAA	TTG	GAT	CCG								

--- 2 Aug - 1990 ----- PC/Gene -----

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## FIG. 6A

\*\*\*\*\*  
 \* TRANSLATION OF A NUCLEIC ACID SEQUENCE \*  
 \*\*\*\*\*

Done on DNA sequence EDEL22.

DE E/DEL virus, vero cells adapted

Total number of bases is: 3180.

Analysis done on the complete sequence.

Done on (absolute) phase(s) : 1.

Using the Universal genetic code.

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10	20	30	40	50														
ATC	GCA	GCG	ATG	ACA	AAC	CTG	CAA	GAT	CAA	ACC	CAC	CAG	ATT	GTT	CCG	TTC	ATA	
---	GAA	TTC	CTC	CTT	CTA	CAA	CCG	TAT	CAT	TGA	TGG	TTA	GTA	3AG	ATC	AGA	CAA	ACG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
60	70	80	90	100														
ATC	GCA	AGC	ATG	ACA	AAC	CTG	CAA	GAT	CAA	ACC	CAC	CAG	ATT	GTT	CCG	TTC	ATA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
110	120	130	140	150	160													
CGG	AGC	CTT	CTG	ATG	CCA	ACA	ACC	GGA	CGA	CCG	GCG	TCC	ATT	CCG	GAC	ACC	CTG	
Arg	Ser	Leu	Leu	MET	Pro	Thr	Thr	Gly	Pro	Thr	Gly	Pro	Ala	Ser	Ile	Pro	Asp	

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FIG. 6B

170	180	190	200	210
GAG CAC ACT CTC AGG TCA GAG ACC TCG ACC TAC AAT TTG ACT GTG GGG GAC	Glu His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr Val Gly Asp			
220	230	240	250	260
GGG TCA CGG CTA ATT GTC TTT CCT GGA TTC CCT GGC TCA ATT GTG GGT				
ACA GGG TCA CGG CTA ATT GTC TTT CCT GGA TTC CCT GGC TCA ATT GTG GGT	Thr Gly Ser Gly Leu Ile Val Phe Pro Gly Phe Pro Gly Ser Ile Val Gly			
280	290	300	310	320
TAC ACA CTG CAG AGC AGT GGG AAC TAC AAG TTC GAT CAG ATG CTC CTG				
GCT CAC TAC ACA CTG CAG AGC AGT GGG AAC TAC AAG TTC GAT CAG ATG CTC CTG	Ala His Tyr Thr Leu Gln Ser Ser Gly Asn Tyr Lys Phe Asp Gln MET Leu Leu			
330	340	350	360	370
GGC CAG AAC CTA CCG GCC AGC TAC AAC TAC TGC AGG CTA GTG AGT CGG AGT				
ACT GGC CAG AAC CTA CCG GCC AGC TAC AAC TAC TGC AGG CTA GTG AGT CGG AGT	Thr Ala Gln Asn Leu Pro Ala Ser Tyr Asn Tyr Cys Arg Leu Val Ser Arg Ser			
380	390	400	410	420
TCA AGG AGC ACA CTC CCT GGT GGC GTR TAT GCA CTA AAC GGC ACC				
CTC ACA GTA AGG TCA AGC ACA CTC CCT GGT GGC GTR TAT GCA CTA AAC GGC ACC	Leu Thr Val Arg Ser Ser Thr Leu Pro Gly Gly Val Val Gly Gly Asn Gly Ser			

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*FIG. 6C*

440	ATA AAC GCC GTG ACC TTC CAA GGA AGC CTG AGT GAA CTG ACA GAT GTT AGC TAC	450	Ile Asn Ala Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr	460		470		480	
490	AAC GGG TTG ATG TCT GCA ACA GCC AAC ATC AAC GAC AAA ATT GGG AAC GTC CTA	500	Asn Gly Leu MET Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu	510		520		530	
540									
550	GTA GGG GAA GGG GTA ACC GTC CTC AGC TTA CCC ACA TCA TAT GAT CTT CCG TAT	560	Val Gly Glu Val Thr Val Leu Ser Pro Thr Ser Tyr Asp Leu G1Y Tyr	570		580		590	
600	GTG AGG CTT GGT GAC CCC ATA CCC GCT ATA GGG CTT GAC CCA AAA ATG GTA GCA	610	Val Arg Leu Gly Asp Pro Ile Pro Ala Ile G1Y Leu Asp Pro Lys MET Val Ala	620		630		640	
650	ACA TGT GAC AGC AGT GAC AGG CCC AGA GTC TAC ACC ATA ACT GCA GCC GAT AAT	660	Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile Thr Ala Ala Asp Asn	670		680		690	
700									

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**FIG. 6D**

TAC	CAA	TTC	TCA	TCA	CAG	TAC	CAA	ACA	GGT	GGG	GTA	ACA	ATC	ACA	CTG	TTC	TCA																										
Tyr	Gln	Phe	Ser	Gln	Tyr	Gln	Thr	Gly	Gly	Val	Thr	Ile	Thr	Ile	Leu	Phe	Ser																										
710	720	730	740	750	760	770	780	790	800	810	820	830	840	850	860	870	880	890	900	910	920	930	940	950	960	970																	
Ala	Asn	Ile	Asp	Ala	Ile	Asn	Thr	Ser	Leu	Val	Gly	Ala	Thr	Ile	Tyr	Leu	Ile	Gly	Ala	Asn	Asn	Ala																					
GCC	AAC	ATT	GAT	GCC	ATC	ACA	AGT	CTC	AGC	GTT	GGG	GGA	GAG	CTC	GTG	TTC	AAA	ACA	GCC	GGC	CTT	ATA	GGC	TTT	GAT	AGC	GTC	CAA	AGC	CTT	GTC	AGC	GTC										
Ala	Asn	Ile	Asp	Ala	Ile	Asn	Ile	Asp	Ala	Ile	Asn	Thr	Ser	Leu	Val	Gly	Ala	Thr	Ile	Ala	Thr	Ala	Thr	Ile	Ala	Asn	Ala																

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**FIG. 6E**

980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210	1220	1230	1240	
CCA ATC ACA TCC ATC ATA CTG GAG ATA GTG ACC TCC AAA AGT GAT GGT CAG GCA					GGG GAA CAG ATG TCA TGG TCG GCA AGT GGG AGC CTA GCA GTG ACG ATC CAT GGT						GGC AAC TAT CCA GGA GCC CTC CGT CCC GTC ACA CTA GTG GCC TAC GAA AGA GTG						GGC GCA ACA GGA TCT GTC GTC GCT GGG GTG AGC AAC TTC GAG CTG ATC CCA						GGC GCA ACA GGA TCT GTC GTC GCT GGG GTG AGC AAC TTC GAG CTG ATC CCA				
Pro Ile Thr Ser Ile Ile Leu Glu Ile Val Thr Ser Lys Ser Asp Gln Gln Ala					Gly Glu Gln MET Ser Trp Ser Ala Ser Gly Ser Gly Ser Gly Ser Gly						Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val Ala Tyr Glu Arg Val						Gly Asn Tyr Val						Gly Asn Pro Glu Ala Leu Asn Lys Asn Leu Val Thr Glu Tyr Gly Arg Phe Asp Pro Gly				

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**FIG. 6F**

1250		1260		1270		1280		1290										
GCC	ATG	AAC	TAC	ACG	AAA	TTG	ATA	CTG	AGT	GAG	AGG	GAC	CAC	CTT	GGC	ATC	AAG	
Ala	MET	Asn	Tyr	Thr	Lys	Leu	Ile	Leu	Ser	Glu	Arg	Asp	His	Leu	Gly	Ile	Lys	
1300		1310		1320		1330		1340		1350								
ACC	GTC	TGG	CCA	ACA	AGG	GAG	TAC	ACT	GAC	TTT	CGT	GAG	TAC	TTC	ATG	GAG	GTG	
Thr	Val	Trp	Pro	Thr	Arg	Glu	Tyr	Thr	Asp	Arg	Glu	Arg	Glu	Tyr	Phe	MET	Glu	Val
1360		1370		1380		1390		1400										
GCC	GAC	CTC	AAC	TCT	CCC	CTG	AAG	ATT	GCA	GGA	GCA	TTT	GGC	TTC	AAA	GAG	ATA	
Ala	Asp	Leu	Asn	Ser	Pro	Leu	Ile	Ala	Gly	Ala	Phe	Gly	Phe	Lys	Asp	Ile		
1410		1420		1430		1440		1450		1500								
ATC	CGG	ATA	AGG	AGG	ATA	GCT	GTA	CCG	GTG	GTC	TCT	ACA	TTG	TTC	CCA	CCT		
Ile	Arg	Ala	Ile	Arg	Ile	Arg	Ile	Ala	Val	Pro	Val	Ser	Thr	Leu	Phe	Pro	Pro	
1460		1470		1480		1490											1510	
GCC	GCT	CCT	CTA	GCC	CAT	GCA	ATT	GGG	GAA	GGT	GTA	GAC	TAC	CTA	CTG	GGC	GAT	
Ala	Ala	Ala	Pro	Leu	Ala	His	Ala	Ile	Gly	Glu	Gly	Val	Asp	Tyr	Leu	Gly	Asp	

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*FIG. 6G*

GAG	GCA	CAG	GCT	GCT	TCA	GGA	ACC	GCT	CGA	GCC	GCG	TCA	GGA	AAA	GCA	AGG	GCT									
Glu	Ala	Gln	Ala	Ala	Ser	Gly	Thr	Ala	Arg	Ala	Ala	Ser	Gly	Lys	Ala	Arg	Ala									
1520	1530	1540	1550	1560	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700	1710	1720						
GCC	TCA	GGC	CGC	ATA	AGG	CAG	CTG	ACT	CTC	GCC	GAC	AAG	GGG	TAC	GAG	GTA	GTC	GCC	GAC	GCG	ATT	CTT	GCT			
Ala	Ser	Gly	Ile	Arg	Ile	Arg	Gln	Leu	Thr	Leu	Ala	Asp	Lys	Gly	Tyr	Glu	Val	Pro	Asn	Pro	Val	Asp	Gly	Ile	Leu	Ala
GTC	GCG	AAT	CTA	TTC	CAG	GTG	CCC	CAG	AAT	CCC	GTA	GTC	GAC	GGG	ATT	CTT	GCT									
Val	Ala	Asn	Leu	Phe	Gln	Val	Pro	Gln	Pro	Val	Asn	Pro	Val	Asp	Gly	Ile	Leu	Ala								
1730	1740	1750	1760	1770	1780																					
GCC	ACG	CTA	TTC	CCT	GTG	GTC	ATT	ACG	ACA	GTG	GAA	GAC	GCC	ATG	ACA	CCC	AAA									
Ala	Thr	Leu	Phe	Pro	Val	Val	Ile	Thr	Thr	Val	Glu	Asp	Ala	MET	Thr	Pro	Lys									

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## FIG. 6H

GCA	CTG	AAC	AGC	AAA	ATG	TTT	GCT	GTC	ATT	GAA	GGC	GTG	CGA	GAA	GAC	CTC	CAA
Ala	Leu	Asn	Ser	Lys	MET	Phe	Ala	Val	Ile	Glu	Gly	Val	Arg	Glu	Asp	Leu	Gln
1790																	
1800																	
1810																	
1820																	
1830																	
1840																	
1850																	
1860																	
1870																	
1880																	
1890																	
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1910																	
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1930																	
1940																	
1950																	
1960																	
1970																	
1980																	
1990																	
2000																	
2010																	
2020																	
2030																	
2040																	
2050																	

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**FIG. 6I**

2060	2070	2080	2090	2100	2110	2120	2130	2140	2150	2160
CGA CCC AAA GTC CCC ATC CAT GTG GCC ATG ACG GGA GCC CTC AAC GCT TGT GGC					GAG ATT GAG AAA ATA AGC TTC AGA AGC ACC AAG CTC GCC ACC GCA CAC CGG CGG CTT					
Arg Pro Lys Val Pro Ile His Val Ala MET Thr GLY Ala Leu Asn Ala Cys G1y					Glu Ile Glu Lys Ile Ser Phe Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu					
2170	2180	2190	2200	2210	2220	2230	2240	2250	2260	2270
GGC CTC AAG TTC GCT GGT CCC GGA GCA TTC GAT GTA AAC ACC GGG CCC AAC TGG					Gly Leu Lys Leu Ala Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp					
2280	2290	2300	2310	2320						
TAC CTC AAC CTT CCA TAC CTT CCA CCC AAT GCA GGA CGC CAG TAC CAC CTT GCC					Tyr Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu Ala					

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FIG. 6.J

2330	ATG GCT GCA TCA GAG TTT AAA GAG ACC CCT GAA CTC GAC AGC GCC GT	2330	AGA GCC 2370
2330	MET Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala Val Arg Ala	2330	
2380	ATG GAA GCA GCA GGC AAT GTG GAC CCA CTG TTC CAA TCT GCA CTC AGT GTG TTC	2380	
2380	MET Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser Ala Leu Ser Val Phe	2380	
2440	ATG TGG CTG GAA GAG AAT GGG ATT GTG GCT GAC ATG GCC AAT RTC GCA CTC AGC	2440	
2440	MET Trp Leu Glu Glu Asn Gly Ile Val Ala Asp MET Ala Asn Phe Ala Leu Ser	2440	
2490	GAC CCG AAC GCC CAT CGG ATG CGA AAT TTT CTG GCA AAC GCA CCA CAA GCA GGC	2490	
2490	Asp Pro Asn Ala His Arg MET Arg Asn Phe Leu Ala Asn Ala Pro Gln Ala Gly	2490	
2540	AGC AAG TCG CAA AGG GCC AAG TAC GGG ACA GCA GGC TAC GGA GTG GAG GCC CGG	2540	
2540	Ser Lys Ser Gln Arg Ala Lys Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg	2540	

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*FIG. 6K*

2600	GGC CCC ACA CCA GAG GAA GCA CAG AGG GAA AAA GAC ACA CGG ATC TCA AAG AAG	2610	Gly Pro Thr Pro Glu Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys	2620		2630		2640	
2650	ATG GAG ACC ATG GGC ATC TAC TTT GCA ACA CCA GAA TGG GTA GCA CTC AAT GGG	2660	MET Glu Thr MET Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly	2670		2680		2690	
2710	CAC CGA GGG CCA AGC CCC GGC CAG CTA AAG TAC TGG CAG AAC ACA CGA GAA ATA	2720	His Arg Gly Pro Ser Pro Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu Ile	2730		2740		2750	
2760	CCG GAC CCA AAC GAG GAC TAT CTA GAC TAC GTG CAT GCA GAG AAG AGC CGG TTG	2770	Pro Asp Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys Ser Arg Leu	2780		2790		2800	
2810	GCA TCA GAA GAA CAA ATC CTA AAG GCA GCT ACG TCG ATC TAC GGG GCT CCA GGA	2820	Ala Ser Glu Glu Gln Ile Leu Lys Ala Ala Thr Ser Ile Tyr Gly Ala Pro Gly	2830		2840		2850	

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*FIG. 6L*

2870	2880	2890	2900	2910	2920	2930	2940	2950	2960	2970
CAG GCA GAG CCA CCC CAA GCT TTC ATA GAC GAA GTT GCC AAA GTC TAT GAA ATC					AAC CAT GGA CGT CCT AAC CAA GAA CAG ATG AAA GAT CTG CTC TTG ACT GCA					
Gln Ala Glu Pro Gln Ala Pro Pro Gln Ala Phe Ile Asp Glu Val Ala Lys Val Tyr Glu Ile					Asn His Gly Arg Gly Pro Asn Gln Glu Gln MET Lys Asp Leu Leu Thr Ala					
2980	2990	3000	3010	3020	3030	3040	3050	3060	3070	3080
ATG GAG ATG AAG CAT CGC AAC CCC AGG CGG GCT CCA CCA AAG CCC AAG CCA AAA					CCC AAT GCT CCA ACA CAG AGA CCC CCT GGT CGG CTG GGC CGC TGG ATC AGG ACC					
MET Glu MET Lys His Arg Asn Pro Arg Ala Pro Pro Lys Pro Lys Pro Lys					Pro Asn Ala Pro Thr Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr					

FIG. 6M

3140                    3150                    3160                    3170                    3180  
GCA GGC GTG GAC ACC AAT TCG GCC TTA CAA CAT CCC AAA TTG GAT CCG  
====24-AUG-1992=====PC/GENE=====

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FIG. 7A

VIRUSES	AMINO ACID RESIDUE NUMBER IN VP2																			
	5	74	76	80	213	222	239	242	249	253	254	258	263	264	269	270	272	277	279	
G1S	Gln	Leu	Ser	Tyr	Asp	Thr	Ser	Val	Lys	His	Ser	Gly	Leu	Ile	Ser	Ala	Ile	Asn	"	
SD326	"	"	"	"	"	"	Asn	Thr	"	"	"	"	"	"	"	"	"	"	"	
E/DEL	"	"	"	"	"	"	Asp	Pro	"	"	Gln	Gly	"	"	"	"	"	"	"	
D78	"	"	"	Gly	"	"	Asp	"	"	"	"	"	"	"	"	"	"	"	"	
Cu-1	"	"	"	Ser	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	
PBG98	"	"	"	"	"	"	"	"	"	"	Arg	"	"	"	"	"	"	"	"	
52/70	"	"	"	"	"	"	"	"	Ile	Gln	Gln	"	"	"	"	"	"	"	"	
STC	"	"	"	"	"	"	Leu	"	Val	"	"	"	"	"	Phe	"	"	"	"	
002-73	Ser	Met	"	Tyr	"	"	Asn	"	"	"	"	"	"	Asn	Leu	Val	"	"	Thr	

FIG. 7B

VIRUSES	AMINO ACID RESIDUE NUMBER IN VP2																			
	280	284	286	279	299	305	311	312	318	320	321	323	326	328	330	332	333	330	332	333
G1S	Asn	Thr	Pro	Asn	Ile	Glu	Ile	Gly	Glu	Glu	Glu	Glu	Leu	"	"	"	"	Ser	Ser	Ser
SD326	"	Ala	"	Ile	"	"	"	Lys	"	Asp	Gln	Ala	Glu	"	"	"	"	"	Ser	Asn
E/DEL	"	"	"	Thr	"	"	"	Glu	"	Gly	"	"	Asp	"	"	"	"	"	"	"
D78	"	"	"	Ser	"	"	"	"	"	"	"	"	"	"	"	"	"	Leu	"	"
Cu-1	"	"	"	Pro	"	"	"	"	"	"	"	"	"	"	"	"	"	Ser	"	"
PBG98	"	Thr	"	Asn	Ala	"	"	"	"	Lys	"	Glu	"	"	"	"	"	"	Arg	"
52/70	"	"	"	Ser	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
STC	"	"	"	Val	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	Asn
002-73	"	"	"	Asn	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"



C (continuation). DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Description of document, with indication, where appropriate, of the relevant passages Relevant to claim No.
14-17	JOURNAL OF GENERAL Virology, Volume 70, issued 1989, K.J. Fahy et al., "A Conformational Immunoogen on VP-2 of Infectious Bursal Disease Virus that Induces Virus-Neutralizing Antibodies That Passively Protect Chickens", pages 1473-1481, see entire document.

INTERNATIONAL SEARCH REPORT

International application No.  
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A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (6):

A61K 35/76, 39/12, 39/395; C07K 14/005, 16/08; C12N 1/21, 5/10, 15/33

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

424/159.1, 185.1, 186.1, 204.1; 435/320.1, 252.3; 530/350, 388.3, 397, 402, 403; 935/10, 12

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